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THE INFLUENCE OF SULFHYDRYL GROUPS AND THEIR INHIBITORS ON THE DISTRIBUTION OF RADIOCOBALT IN THE ORGANS AND INTRACELLULAR ORGANELLES OF THE MOUSE

By

LAURENCE S. MAYNARD

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THE INFLUENCE OF SULFHYDRYL GROUPS AND THEIR INHIBITORS ON THE DISTRIBUTION OF RADIOCOBALT IN THE ORGANS AND INTRACELLULAR ORGANELLES OF THE MOUSE*

By

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INTRODUCTION

Sulfhydryl groups and their inhibitors have intrigued biochemists and histochemists for some time, and the relationship of these reactive groups to certain enzyme systems is now well established. The studies reported in this paper appear to demonstrate that, contrary to preconceived expectations, the sulfhydryl-containing amino acid L-cysteine has inhibitory effects on the accumulation of cobalt by living tissues, while iodoacetate, a sulfhydryl inhibitor, seems to potentiate such accumulation. Furthermore, the effect of these substances on the uptake of cobalt by liver mitochondria *in vivo* is far less impressive than is the case with intact organs. This may be interpreted as indicating a fundamental difference in the mitochondrial membrane as compared to the cell membrane, at least as far as cobalt transport is concerned.

In recent years cobalt has been studied primarily in the form of its compound Vitamin B_{12}[1,2] while, in comparison, the behavior of inorganic cobalt has been neglected. This is understandable, but perhaps unfortunate, since cobalt, a divalent metal of the transition series of elements, has certain features of metabolism and transport that are of interest to students of cellular permeability and active transport. Studies of manganese metabolism[3,4] and of iron metabolism[5,6] reveal that these transition elements have some suggestive similarities and dissimilarities of behavior compared to those of cobalt, as described in this paper. The fact that Co, unlike Mn and Fe, has never been shown to change its valence state within living tissues may have some bearing on the dissimilarities.

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Several workers have studied the organ distribution of cobalt,[7-10] and the consensus is that the highest concentrations of the metal are found in the liver, kidneys, pancreas, and spleen. Parenterally administered cobalt is excreted primarily in the urine, and the high levels of fecal cobalt found after oral dosage are presumed to be due to incomplete absorption of this substance by the intestinal tract.[7,11]

The kinetics of cobalt absorption from the peritoneal cavity into the blood and from the blood into the tissues have been followed by means of a blood-sampling technique. It appears that each of these absorption processes can be described by a simple exponential curve, absorption from the peritoneal cavity being the slower of the two processes. In connection with the present investigation it is of significance that the use of cobalt-histidine and cobalt-cysteine complexes did not alter the basic pattern of the kinetics of absorption.[12]

In this study the effects on cobalt uptake of a number of compounds, including SH inhibitors, SH-group-containing substances, nonspecific inhibitors, and some miscellaneous physiologically active compounds were studied *in vitro* in a series of survey experiments. As a result of this survey, two compounds, L-cysteine and sodium iodoacetate, were selected for more intensive study *in vivo*.

METHODS

In Vitro Studies

Mouse kidney was sliced in a Stadie-Riggs tissue slicer, blotted, and weighed, and single slices were placed in Warburg vessels containing 2.5 ml. of Tris-buffered Ringer's solution (*p*H 7.4), and 0.5 ml. of Co^{60} solution was placed in each side arm. The vessels were gassed with 0_2 and allowed to reach temperature equilibrium for 15 minutes at 37° C; the Co^{60} was tipped in, and timing of the run was begun. Consumption of 0_2 was followed solely as evidence that the tissue slice was living and respiring satisfactorily. At the end of one hour a sample of the substrate was withdrawn and counted in a well-type scintillation counter to determine the percentage of the radiocobalt removed from the solution by the tissue slice. Corrections were made for the differing weights of the tissue slices. This basic procedure was modified by the addition to the substrate of various compounds to determine their effect on cobalt uptake. All experiments were performed in duplicate or triplicate.

In Vivo Studies

Four groups of 6 to 8 white male mice of approximately the same weight were selected and fed on a standard diet of Purina laboratory chow *ad libitum*. Each mouse received an injection of 2.5 μc. of Co^{60} intraperitoneally, dissolved in 0.25 ml. of Tris-buffered Ringer's solution (*p*H about 7.4). The total amount of cobalt injected was 0.65 μg. The groups were handled as follows:

(1) Twenty-four hour controls: sacrificed 24 hours after injection and organs removed.

(2) Seventy-two hour controls: sacrificed at 72 hours.

(3) Iodoacetate: 0.5 μM of Na iodoacetate per gm. of mouse (0.5 μM per 0.01 ml. of solution given intraperitoneally (I.P.) a few minutes before the Co^{60}; sacrificed at 24 hours.

(4) L-cysteine: 0.5 μM of cysteine per gm. of mouse (0.5 μM per 0.01 ml. of solution) given I.P. as a freshly made-up solution a few minutes before injection of Co^{60}; sacrificed at 24 hours.

All mice were sacrificed by light chloroform anesthesia, followed by rapid exsanguination and dissection; removal of the desired tissues was accomplished rapidly in a cold room at 3 to 5° C. The organs were placed in small glass vials, weighed, and counted in a well-type scintillation counter.[3] A portion of the liver was set aside, weighed, and used for intracellular distribution studies.

For these intracellular distribution studies, approximately 1 gm. of minced liver from each mouse was made up to 10 ml. with 0.44 M sucrose solution, homogenized in a glass homogenizer with a Teflon pestle, and fractionated by differential centrifugation. The sucrose concentration and fractionation procedure were those recommended by Witter *et al.*[13], as best preserving the morphologic integrity of the mitochondria. Three fractions were obtained: nuclear, mitochondrial, and supernatant (containing microsomes). The total nitrogen content of each fraction and of the unfractionated homogenate was determined by means of a mercury-catalyzed Kjeldahl digestion,[14] followed by a direct nesslerization procedure. Co^{60} assay was carried out on 2 ml. samples of each fraction in the well-type scintillation counter. Results were expressed both as percentages of total liver Co^{60} found in each fraction, and as concentration; that is, Co^{60} per μM of nitrogen in each fraction, with the whole-liver concentration set equal to 1.000.

RESULTS

In Vitro Studies

The effects of the various compounds studied in the *in vitro* system on cobalt uptake may be summarized as follows:

(1) Compounds increasing Co^{60} uptake: iodoacetate 10^{-4} M, *p*-chloromercuribenzoate 10^{-5} M, quinone 10^{-4} M, and Hg^{++} 10^{-3} M.

(2) Compounds having no effect on Co^{60} uptake: fluoride 10^{-2} M, glutamate 10^{-2} M, thyroxine 10^{-5} M and 10^{-6} M, 2 : 4 dinitrophenol 10^{-4} M to 10^{-6} M.

(3) Compounds decreasing Co^{60} uptake: L-cysteine 10^{-3} M, glutathione 10^{-3} M, Mn^{++} 10^{-2} M, and CN^- 10^{-4} M (this effect proved to be due to complex-ion formation as measured by Schubert's technique.[15]

It was also observed that the effects of *p*-chloromercuribenzoate were completely reversed by the addition of excess cysteine or glutathione.

Since these survey experiments indicated that four SH inhibitors increased the uptake of Co^{60}, while two SH-containing compounds decreased it, it was decided to study representatives of each class of compound more thoroughly in an *in vivo* type of experiment. Because of its high solubility and relatively low toxicity, iodoacetate was selected as the SH inhibitor and cysteine was used as the SH-containing substance.

In Vivo Studies

Organ distribution studies. TABLE 1 summarizes these results in terms of percentage of injected Co^{60} taken up per gram of tissue, and TABLE 2 as percentage of Co^{60} dose taken up by the whole organ. Since the blood levels were profoundly modified by the use of cysteine and iodoacetate, TABLE 1 also includes the liver/blood ratio to permit comparison of the degree of saturation of liver tissue independent of varying blood levels. Standard deviations of the mean were calculated, and tests for statistical significance of the differences between means were made by the *t* test for small samples.[16]

The 24-hour controls differ significantly (P: less than 1 per cent) from the other three groups in respect to liver and blood levels, and for most of the other tissues as well.

Intracellular distribution studies. This series of experiments is summarized in TABLE 3. Application of the *t* test to these data demonstrates that the mitochondrial concentration of Co^{60} in the 72-hour controls and in the cysteine groups is significantly higher than in the 24-hour controls (P: 0.6 per cent and 1.0 per cent, respectively). Some discrepancies between the crude percentages and the concentrations will be noted. These will be resolved by consideration of the nitrogen content of the fractions. It is obvious that less mitochondrial nitrogen is found in the iodoacetate and cysteine groups, perhaps because of toxic effects of these substances (the animals given iodoacetate appeared sick, remained quiet, and seemed to be somewhat anoxic).

TABLE 1

UPTAKE OF COBALT^{60} BY DIFFERENT ORGANS: PERCENTAGE OF DOSE PER GRAM OF TISSUE AS MODIFIED BY VARYING TIME OF SACRIFICE OR BY GIVING IODOACETATE OR CYSTEINE INTRAPERITONEALLY

Organ	*Control group 24 hours after dose*	*Control group 72 hours after dose*	*Iodoacetate 0.5 μM /gm. 24 hours after dose*	*Cysteine 0.5 μM /gm. 24 hours after dose*
Liver	2.52 ±0.38*	1.27 ±0.19*	3.96 ±0.25*	0.73 ±0.11*
Kidneys	2.30 ±0.11	0.93 ±0.06	3.15 ±0.27	0.53 ±0.06
Pancreas	2.11 ±0.25	0.76 ±0.10	2.46 ±0.16	0.22 ±0.04
Spleen	0.89 ±0.07	0.30 ±0.03	1.56 ±0.12	0.13 ±0.02
Heart	1.91 ±0.21	0.93 ±0.07	1.49 ±0.08	0.34 ±0.06
Muscle	0.38 ±0.04	0.11 ±0.01	0.59 ±0.07	0.05 ±0.01
Lungs	1.64 ±0.15	0.55 ±0.04	2.86 ±0.32	0.28 ±0.04
Brain	0.18 ±0.02	0.10 ±0.01	0.14 ±0.01	0.03 ±0.01
Blood	2.47 ±0.13	0.43 ±0.01	3.88 ±0.42	0.21 ±0.04
Liver/blood	1.01 ±0.13	3.07 ±0.515	1.08 ±0.108	3.63 ±0.469
Average weight of mice in group	23.7 gm. ±1.98	25.0 gm. ±1.61	31.8 gm. ±0.82	24.8 gm. ±1.44

*The value after the ± is the standard deviation of the mean.

TABLE 2

UPTAKE OF COBALT^{60} BY DIFFERENT ORGANS: PERCENTAGE OF DOSE IN WHOLE ORGAN AS MODIFIED BY VARYING TIME OF SACRIFICE OR BY GIVING IODOACETATE OR CYSTEINE INTRAPERITONEALLY

Organ	*Control group 24 hours after dose*	*Control group 72 hours after dose*	*Iodoacetate 0.5 μM /gm. 24 hours after dose*	*Cysteine 0.5 μM /gm. 24 hours after dose*
Liver	3.49 ±0.38*	2.01 ±0.28*	5.69 ±0.28*	1.18 ±0.16*
Kidneys (2)	0.90 ±0.08	0.41 ±0.03	1.57 ±0.20	0.24 ±0.02
Pancreas	0.52 ±0.05	0.23 ±0.03	0.84 ±0.08	0.07 ±0.01
Spleen	0.15 ±0.03	0.05 ±0.01	0.11 ±0.01	0.03 ±0.01
Heart	0.20 ±0.02	0.11 ±0.01	0.23 ±0.01	0.05 ±0.01
Lungs	0.27 ±0.02	0.10 ±0.01	0.60 ±0.09	0.06 ±0.01
Brain	0.08 ±0.01	0.04 ±0.002	0.06 ±0.01	0.01 ±0.001
Average weight of mice in group	23.6 gm. ±1.98	25.0 gm. ±1.61	31.8 gm. ±0.82	24.8 gm. ±1.44

*The value after the ± is the standard deviation of the mean.

DISCUSSION

Most readily apparent is the profound effect of iodoacetate and cysteine on the over-all uptake of cobalt by the tissues. In comparison to the 24-hour controls, the sulfhydryl inhibitor, iodoacetate, markedly increases the uptake of Co^{60} by liver and most of the other organs (TABLES 1 and 2), while cysteine even more markedly decreases the uptake. This *in vivo* effect on cobalt is analogous to the *in vitro* effect observed by Saltman *et al*[6] in studies of iron metabolism.

The cysteine-treated mice at 24 hours closely resemble the controls at 72 hours. It may be objected that cysteine forms a relatively weak complex with cobalt, and therefore all that we are observing is the well-demonstrated phenomenon that some complexing agents will increase the excretion rate of various metals.[4] However, iodoacetate has no measurable complexing effect when tested by the ion exchange method;[15] its sole action is one of inactivation of SH groups by alkylation.[17] Nevertheless, it produces a definite effect that is the reverse of that produced by cysteine. It seems reasonable, then, to assume that the SH groups of cysteine are specifically responsible for its effects.

The question now arises: Do SH groups decrease the uptake of cobalt by tissues by lowering the rate of absorption, or do they increase the rate of excretion? This series of experiments does not answer this question directly, but the resemblance of the cysteine group to the controls at 72 hours suggests that the key factor is an increase in the rate of excretion.

However, even if we set aside the question of the exact mode of action of cysteine, since it has not yet been established, the fact remains that, with cobalt, cysteine markedly depresses the accumulation of this metal in tissues, while iodoacetate, an SH inhibitor, enhances such uptake to nearly an equal degree. This state of affairs is contrary to what one would expect according to the usual theories of active transport across cell membranes.[18]

Turning to the intracellular distribution studies (TABLE 3), it is obvious that mitochondria do concentrate cobalt to a level above that of the cell as a whole, just as they do with manganese.[3] This phenomenon is most conspicuous in the 72-hour control group. This suggests that mitochondria retain the cobalt more tenaciously than do the other elements of the cell.

It will be noted also that, although cysteine does increase somewhat the Co^{60} concentration of mitochondria (TABLE 3), paralleling to an extent the 72-hour control group, the parallel is by no means as marked, nor is the cysteine effect as extensive, as that seen in comparing cobalt

TABLE 3

INTRACELLULAR DISTRIBUTION OF COBALT^{60} LIVER-CELL FRACTIONATION STUDIES, AS MODIFIED BY VARYING TIME OF SACRIFICE OR BY GIVING IODOACETATE OR CYSTEINE INTRAPERITONEALLY

	Control group 24 hours after dose			*Control group 72 hours after dose*			*Iodoacetate 0.5 μM /gm. 24 hours after dose*			*Cysteine 0.5 μM /gm. 24 hours after dose*		
Fraction	*Co^{60} in fraction Crude %*	*% total N in fraction*	*Conc.* Co^{60}*	*Co^{60} in fraction Crude %*	*% total N in fraction*	*Conc.* Co^{60}*	*Co^{60} in fraction Crude %*	*% total N in fraction*	*Conc.* Co^{60}*	*Co^{60} in fraction Crude %*	*% total N in fraction*	*Conc.* Co^{60}*
Nuclei	14.72% ±1.31†	18.76% ±1.19	0.780 ±0.023†	17.02% ±1.91	19.55% ±1.44	0.860 ±0.037	13.99% ±0.98	20.40% ±1.18	0.684 ±0.020	11.86% ±0.89	15.97% ±0.90	0.747 ±0.47
Mitochondria	33.52% ±0.83	24.07% ±0.22	1.393 ±0.035	44.96% ±1.53	24.97% ±1.16	1.825 ±0.117	25.85% ±1.15	19.43% ±0.96	1.337 ±0.048	30.65% ±1.21	19.38% ±1.22	1.598 ±0.056
Supernatant	50.25% ±1.69	55.44% ±1.06	0.906 ±0.025	36.68% ±1.48	55.22% ±1.63	0.666 ±0.025	56.83% ±1.40	60.04% ±2.79	0.954 ±0.028	56.80% ±1.06	66.52% ±0.93	0.855 ±0.021
Recovery of Co^{60}	98.49% ± 0.34			98.66% ± 0.65			96.63% ± 0.93			99.32% ± 0.51		
Recovery of Tissue N	98.27% ± 0.69			99.74% ± 1.59			99.87% ± 2.49			101.87% ± 1.44		

*Concentrations are expressed as counts/μM of tissue N, with the value for whole, unfractionated liver homogenate set equal to 1.000.
†Value after ± is standard deviation of the mean.

concentrations in tissues or organs. Likewise, iodoacetate has little or no effect on mitochondrial concentration of cobalt compared to the 24-hour control. The effects on crude percentage (TABLE 3) may be dismissed as a toxic effect on the liver cell, with resulting decrease in the total amount of mitochondrial nitrogen.

Consequently, we have a situation in which cysteine and iodoacetate have dramatic and opposed effects on the uptake of cobalt by the tissues of the living mouse, but have little or no effect on the intracellular distribution of the cobalt.

Two hypotheses suggest themselves to explain these results:

(1) Cysteine and iodoacetate do not penetrate the cell membrane readily; therefore they exert little effect on mitochondria.

(2) The mode of cobalt transport through the cell membrane may differ from that through the mitochondrial membrane. SH groups and their inhibitors may influence the former, but not the latter.

The first hypothesis seems unlikely, as cysteine is metabolized by tissue slices, and presumably must enter the cell for this to occur. Whether or not the second hypothesis will stand up in the light of further investigation must be decided by additional studies, now in progress, of the role of sulfhydryl groups in cobalt transport, with special attention devoted to their site of activity.

SUMMARY

(1) Intraperitoneally injected radiocobalt (Co^{60}) is rapidly concentrated by the tissues of the mouse, the highest levels being obtained in the liver, the kidneys, and the pancreas.

(2) Sucrose fractionation of these mouse livers indicates that mitochondria concentrate cobalt to a level exceeding that of the rest of the cell.

(3) Survey experiments performed *in vitro* indicate that several SH inhibitors increase Co^{60} uptake, while several SH-containing substances inhibit this uptake.

(4) Cysteine, an SH-containing amino acid, depresses cobalt uptake in mouse tissues *in vivo*, while iodoacetate, an SH antagonist, increases uptake.

(5) Cysteine and iodoacetate have much less marked effects on intracellular distribution of cobalt than they do on tissue concentration.

(6) It is suggested that the SH groups influence cobalt transport primarily at the cell membrane, and that the lesser effect at the mitochondrial membrane may possibly indicate a fundamental difference in the nature of the two membranes, at least in regard to their handling of cobalt.

References

1. BARBEE, K. W. & B. C. JOHNSON. 1951. Proc. Soc. Exptl. Biol. Med. 76: 720.
2. CHOW, B. F., C. ROSENBLUM, R. H. SILBER, D. T. WOODBURY, R. YAMAMOTO & C. A. LANG. 1951. Proc. Soc. Exptl. Biol. Med. 76: 393.
3. MAYNARD, L. S. & C. G. COTZIAS. 1955. J. Biol. Chem. 214: 831.
4. MAYNARD, L. S. & S. FINK. J. 1956. Clin. Invest. 35: 831.
5. SALTMAN, P., R. D. FISKIN & S. B. BELLINGER, 1956. J. Biol. Chem. 220:741.
6. SALTMAN, P., R. D. FISKIN, S. B. BELLINGER & T. ALEX. 1956. J. Biol. Chem. 220: 751.
7. COPP, D. H. & D. M. GREENBERG. 1941. Proc. Natl. Acad. Sci. 27: 153.
8. COMAR, C. L. & G. K. DAVIS. 1947. J. Biol. Chem. 170: 379.
9. BRAUDE, R., A. A. FREE, J. E. PAGE & E. L. SMITH. 1949. Brit. J. Nutrition. 3: 289.
10. CUTHBERTSON, W. F. J., A. A. FREE & D. M. THORNTON. 1950. Brit. J. Nutrition. 4: 40.
11. GREENBERG, D. M., D. H. COPP & E. M. CUTHBERTSON. 1943. J. Biol. Chem. 147: 747.
12. BERLIN, N. I. & W. SIRI. 1951. Am. J. Physiol. 164: 221.
13. WITTER, R. F., M. L. WATSON & M. A. COTTONE. 1955. J. Biophys. Biochem. Cytol. 1: 127.
14. HILLER, A., J. PLAZIN & D. D. VAN SLYKE.1948. J. Biol. Chem. 176: 1401.
15. SCHUBERT, J. 1952. J. Phys. Chem. 56: 113.
16. TRELOAR, A. E. 1951. Biometric Analysis, an Introduction. : 105-107. Minneapolis, Minn.
17. HUGHES, W. L. 1957. Ann. N. Y. Acad. Sci. 65(5): 454.
18. ROSENBERG, T. 1954. *In* Symposia Soc. Exptl. Biol. No. 8: 27-41.

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